Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 965–968

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Vanilloid and Isovanilloid Analogues as Inhibitors of Methionyl-tRNA and Isoleucyl-tRNA Synthetases

Jeewoo Lee,[a,*] Sang Uk Kang,[a] Su Yeon Kim,[a] Sung Eun Kim,[a] Yeong Joon Jo[b] and Sunghoon Kim[c]

[a]*Laboratory of Medicinal Chemistry, College of Pharmacy, Seoul National University, Shinlim-Dong, Kwanak-Ku, Seoul 151-742, South Korea*

[b]*ImaGene Co., Sung Kyun Kwan University, 300 Chunchundong, Jangangu, Suwon, Kyunggido 440-746, South Korea*

[c]*National Creative Research Initiative Center for ARS Network, Sung Kyun Kwan University, 300 Chunchundong, Jangangu, Suwon, Kyunggido 440-746, South Korea*

Received 24 November 2000; accepted 31 January 2001

Abstract—As aminoacyl adenylate surrogates, a series of methionyl and isoleucyl phenolic analogues containing bioisosteric linkers mimicking ribose have been investigated. Inhibition of synthesized compounds to the aminoacylation reaction by the corresponding *Escherichia coli* methionyl-tRNA and isoleucyl-tRNA synthetases indicated that **18** was found to be a potent inhibitor of isoleucyl-tRNA synthetase. A molecular modeling study demonstrated that in **18**, isovanillate and hydroxamate served as proper surrogates for adenine and ribose in isoleucyl adenylate, respectively.

Introduction

In the preceding communication,[1] we described the structure–activity relationship of ester, and the hydroxamate analogues of methionyl and isoleucyl adenylate, as methionyl-tRNA (MetRS) and isoleucyl-tRNA synthetase (IleRS) inhibitors.

In our continuing effort to find potent aminoacyl-tRNA synthetases (aaRSs) inhibitors, based on the ester analogue of aminoacyl adenylate (**1**) as a lead compound,[2] we modified the adenine and ribose parts with their appropriate bioisosteres, as depicted in Figure 1. Firstly, the adenine moiety was replaced by vanilloid or isovanilloid, in which phenolic hydroxyl and the methoxy group was supposed to mimic 6-NH_2 of adenine as a hydrogen bonding donor, and N-1 of adenine as a hydrogen bonding acceptor, respectively. Secondly, the ribose was substituted by its biosteres, such as acyclic amide and hydroxamate (**2**), dihydroisooxazole (**3**) and dihydrooxazole (**4**). In particular, the hydroxamate group has been attractive in terms of its binding mode, in which carbonyl oxygen is able to mimic the ring oxygen of ribose, and *N*-hydroxyl serves as 2′-hydroxyl of sugar. These ribose surrogates have been successfully utilized for pharmacologically active adenylates in drug design.[3]

In this paper, we describe the synthesis of designed methionyl and isoleucyl vanilloids (or isovanilloids) containing ribose bioisosteres and their inhibitory activities to *Escherichia coli* MetRS and IleRS.

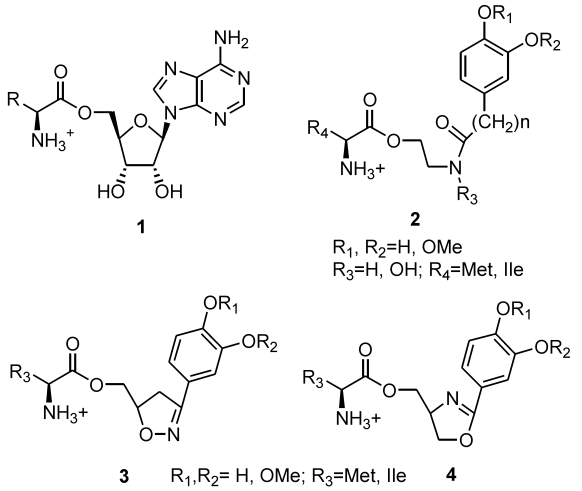
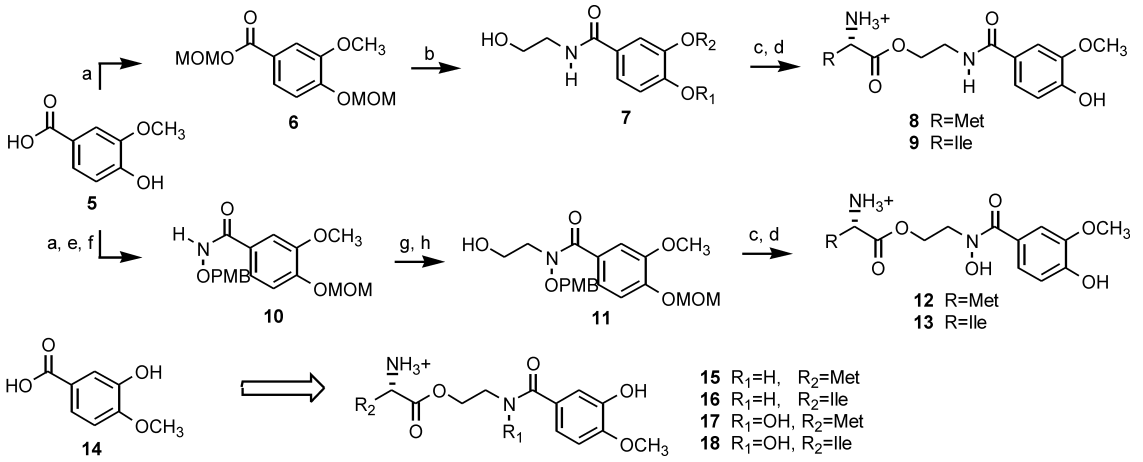


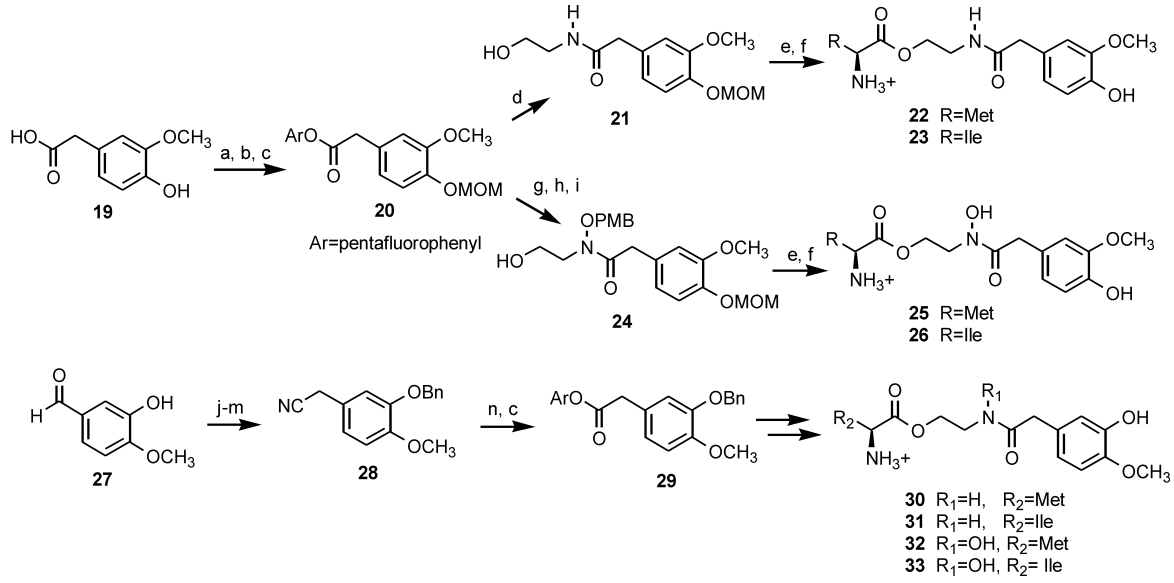
Figure 1.

*Corresponding author. Tel.: +82-2-880-7846; fax: +82-2-888-0649; e-mail: jeewoo@snu.ac.kr

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00096-8



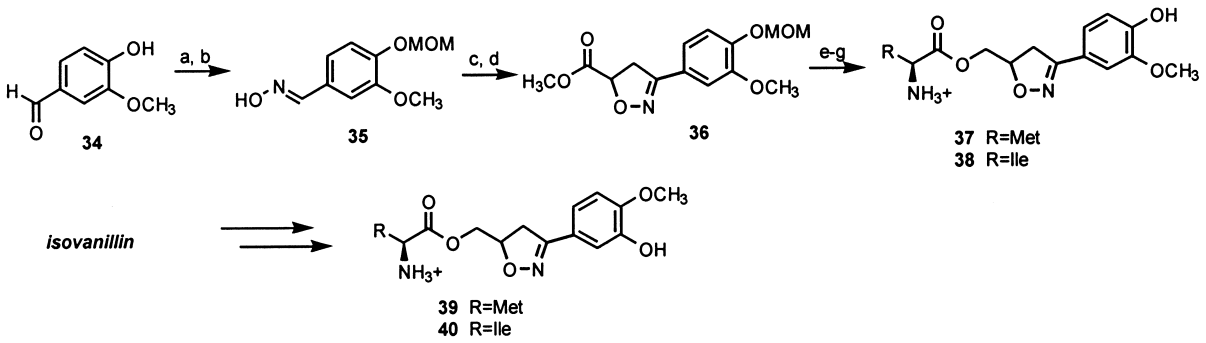
Scheme 1. Reagents and conditions: (a) MOMCl, *i*-Pr_2NEt, DMF, 85%; (b) $NH_2CH_2CH_2OH$, 100 °C, 90%; (c) *N*-Boclle or *N*-BocMet, DCC, DMAP, CH_2Cl_2, 94%; (d) TFA, anisole, 90%; (e) NaOH, THF–H_2O, 85%; (f) *t*-BuOCOCl, 4-MNO, CH_2Cl_2; then $HClNH_2OPMB$, 60%; (g) NaH, $BrCH_2CH_2OTBS$, DMF, 35%; (h) Bu_4NF, THF, 92%.



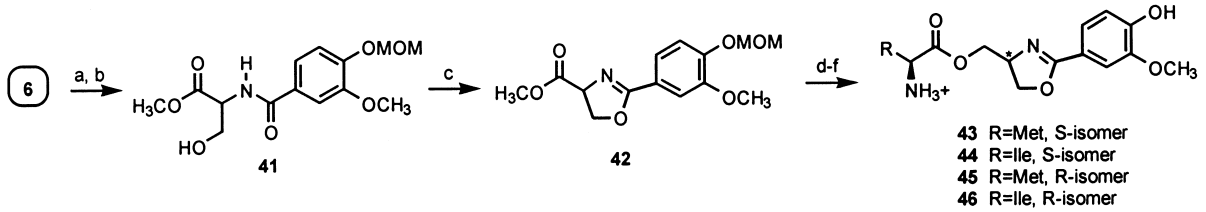
Scheme 2. Reagents and conditions: (a) MOMCl, *i*-Pr_2NEt, DMF; (b) NaOH, THF–H_2O; (c) pentafluorophenol, DCC, DMAP, CH_2Cl_2, 75% in three steps; (d) $NH_2CH_2CH_2OH$, DMF, 95%; (e) *N*-Bocelle or *N*-BocMet, DCC, DMAP, CH_2Cl_2, 93%; (f) TFA, anisole, 92%; (g) NH_2OPMB, DMF, 92%; (h) $HOCH_2CH_2OTBS$, PPh_3, DEAD, THF, 84%; (i) Bu_4NF, THF, 50%; (j) NaH, BnBr, DMF, 95%; (k) $NaBH_4$, MeOH; (l) MsCl, NEt_3, CH_2Cl_2; (m) NaCN, DMF, 20% in three steps; (n) 30% NaOH, reflux, 70%.

Synthesis

The syntheses of vanillate and isovanillate analogues are outlined in Scheme 1. Commercially available vanillic acid (**5**) was protected by the methoxymethyl group on both acid and hydroxyl to give **6**, which reacted directly with 2-aminoethyl alcohol to afford amide **7**. The condensation of alcohol **7** with *N*-Boc methionine or *N*-Boc isoleucine was followed by acidic hydrolysis to provide vanillic amides **8** and **9**, respectively. For the synthesis of vanillic hydroxamate analogues, ester **6** was hydrolyzed and then condensed with *O*-4-methoxybenzylhydroxylamine[4] to give hydroxamate **10**. The *N*-alkylation of **10** with *O*-TBS 2-bromoethanol followed by desilylation afforded alcohol **11**, which was converted into **12** and **13** by following the amide procedure. Isovanillic amide (**15**, **16**) and hydroxamate analogues (**17**, **18**) were prepared from isovanillic acid (**14**) by following the same method described previously. The synthetic sequence employed for homovanillate and homoisovanillate analogues was depicted in Scheme 2. Homovanillic acid (**19**) was converted into the corresponding pentafluoroester **20** by three steps. The condensation of **20** with 2-aminoalcohol gave alcohol **21**, which was then converted into homovanillic amide analogues **22** and **23** by following the sequence of reactions described in Scheme 1. The condensation of **20** with *O*-4-methoxybenzyl hydroxylamine, the Mitsunobu reaction with monoprotected ethylene glycol followed by desilylation, produced alcohol **24**, which was conventionally converted into homovanillic hydroxamate analogues **25** and **26**, respectively. For the synthesis of homoisovanillate analogues, isovanillin was transformed into



Scheme 3. Reagents and conditions: (a) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, 95%; (b) HCl–NH_2OH, $NaHCO_3$, EtOH, 95%; (c) NCS, DMF, 80%; (d) $CH_2{=}CHCO_2CH_3$, NaOCl, CH_2Cl_2, 70%; (e) $LiAlH_4$, ether, 80%; (f) *N*-Boclle, DCC, DMAP, CH_2Cl_2, 92%; (g) TFA, anisole, 97%.



Scheme 4. Reagents and conditions: (a) NaOH, THF–H_2O, 85%; (b) *t*-BuOCOCl, 4-NMO, CH_2Cl_2; then L(or D)-serine methyl ester, 70%; (c) PPh_3, DEAD, THF, 91%; (d) $NaBH_4$, MeOH, 60%; (e) *N*-Boclle (or *N*-BocMet), DCC, DMAP, CH_2Cl_2, 90–92%; (f) TFA, anisole, 90–94%.

the corresponding pentafluoroester **29** by six steps, which was converted into **30**–**33** by following the same sequence used for the synthesis of homovanillate analogues.

The dihydroisooxazole analogues were synthesized as represented in Scheme 3. Aldehyde of vanillin (or isovanillin) was transformed into a dihydroisooxazole derivative **36** by four steps via nitrile oxide 1,3-dipolar addition.[5] The sequence described in Scheme 1 provided the dihydroisooxazoles **37**–**40**. For the synthesis of dihydrooxazole analogues, as shown in Scheme 4, ester **6** was hydrolyzed and then condensed with L(or D)-serine methyl ester to give **41**, which was cyclized into **42** by intramolecular *O*-alkylation. The same method that was described in Scheme 3 with **42** produced the dihydrooxazoles **43**–**46**.

Biological Results and Discussion

Synthesized vanillate and isovanillate analogues were evaluated as inhibitors of *E. coli* MetRS or IleRS. Their inhibitory activities were determined by measuring the decrease of the aminoacylation product, the [^{35}S]methionyl $tRNA^{Met}$ or [^{3}H]isoleucyl $tRNA^{Ile}$ of *E. coli*, in the presence of different chemical concentrations.[6]

Among IleRS inhibitors tested, the isovanillic hydroxamate analogue **18** was found to be the most potent one with $IC_{50} = 4.5\,\mu M$. The fact that its amide surrogate **16** ($IC_{50} = 106\,\mu M$) displayed a 24-fold decrease as inhibitor, indicated that the hydroxyl of hydroxamate played an important role in interacting with enzymes, and probably acted as a mimic of 2′-OH in isoleucyl adenylate as expected. Vanillic hydroxamate **13** ($IC_{50} = 12.8\,\mu M$) with a phenolic hydroxyl at the *para*-position and homoisovanillic surrogates **33** ($IC_{50} = 21\,\mu M$) with a one-carbon elongated hydroxamate, also proved less potent than **18** by ca. 3- and 4.5-fold, respectively. Obviously, the specific interaction of the two hydroxyls of phenolic and hydroxmate in **18** would be required for its inhibitory activity. Dihydroisooxazole (**38**, **40**) and dihydrooxazole analogues (**44**, **46**) showed moderate inhibitory activities ($IC_{50} = 38.6$–$79\,\mu M$) as IleRS inhibitors.

Concerning the MRS inhibitors, although the vanillic hydroxamate analogue **12** appeared to be optimal in this series, most of the compounds displayed moderate inhibition to *E. coli* MetRS ($IC_{50} = 32.5$–$163.5\,\mu M$) (Table 1).

A molecular modeling study of **18** as a potent *E. coli* IleRS inhibitor was conducted by overlapping **18** onto isoleucyl adenylate as a standard. The energy-minimized structure of isoleucyl adenylate was obtained based on reported X-ray structures of several aminoacyl adenylates bound to aminoacyl-tRNA synthetases.[7]

Table 1. Enzyme inhibitory activities of synthesized compounds toward MetRS and IleRS

MRS Inhibitors	*E. coli* MRS (IC_{50}, μM)	IRS inhibitors	*E. coli* IRS (IC_{50}, μM)
8	60	**9**	64.4
12	32.5	**13**	12.8
15	78	**16**	106
17	72	**18**	4.5
22	94	**23**	45
25	128.5	**26**	228
30	163.5	**31**	56.5
32	87	**33**	21
37	58	**38**	79
39	47	**40**	57.6
43	33.5	**44**	38.6
45	39	**46**	50

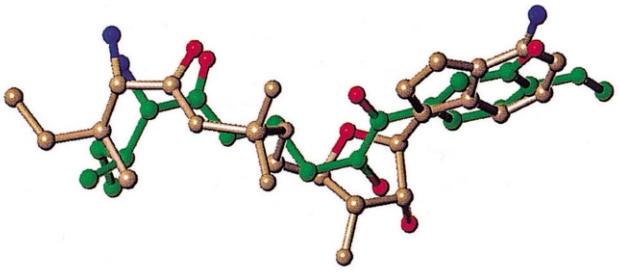


Figure 2. Superposition of **18** (green) on isoleucyl adenylate (wheat): only matched pharmacophores are colored: oxygen (red), nitrogen (blue).

The low-energy structure of **18** was calculated using the SYBYL 6.6 program.[8] We performed a molecular alignment and pharmacophore detection for two energy-minimized molecules using GASP (Genetic Algorithm Similarity Program, Tripos). During the calculation, the conformation of isoleucyl adenylate was fixed as a reference structure. In the result, **18** was aligned to isoleucyl adenylate to match with five pharmacophores (amino and carbonyl in isoleucine, oxygen and 2′-hydroxyl on ribose, and amine of adenine). In the overlapped structure, shown in Figure 2, **18** aligns itself with key pharmacophores of isoleucyl adenylate, providing that isovanillic and hydroxamate moieties are adequate surrogates for adenine and ribose, respectively. The overlay will be useful information for the design of improved related compounds.

In summary, we investigated acyclic surrogates of methionyl adenylate and isoleucyl adenylate as MetRS and IleRS inhibitors having vanilloid (or isovanilloid) and ribose bioisosteres. Isovanillic hydroxamate **18** displayed potent inhibitory activity to *E. coli* IleRS, and its pharmacophores matched well with those of isoleucyl adenylate by molecular modeling. Further investigation is in progress to find more potent and selective inhibitors of aminoacyl-tRNA synthetases based on **18**.

Acknowledgements

This work was supported in part by a grant (HMP-00-CH-15-0014) from the Ministry of Health & Welfare and by the Brain Korea 21 Project to J. Lee, as well as a grant from the National Creative Research Initiatives from Ministry of Science and Technology to S. Kim.

References and Notes

1. Lee, J.; Kang, S. U.; Kim, S. Y.; Kim, S. E.; Jo, Y. J.; Kim, S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 961.
2. Lee, J.; Kang, S. U.; Kang, M. K.; Chun, M. W.; Jo, Y. J.; Kwak, J. H.; Kim, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1365.
3. Kleinman, E. F.; Campbell, E.; Giordano, L. A.; Cohan, V. L.; Jenkinson, T. H.; Cheng, J. B.; Shirley, J. T.; Pettipher, E. R.; Salter, E. D.; Hibbs, T.; DiCapua, F. M.; Bordner, J. *J. Med. Chem.* **1998**, *41*, 266.
4. Kim, M. G.; Jung, J. C.; Sung, M. J.; Choi, Y. K.; An, S. G.; Lee, S.-J.; Yoon, G.-J.; Park, M. H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2077.
5. Carruthers, W. In *Cycloaddition Reactions in Organic Synthesis*; Pergamon: Oxford, 1990; p 285.
6. Jo, Y. J.; Lee, S. W.; Jo, M. K.; Lee, J.; Kang, M.-K.; Yoon, J. H.; Kim, S. J. *Biochem. Mol. Biol.* **1999**, *32*, 547.
7. We extracted and superimposed X-ray structures of aminoacyl adenylates embedded in aminoacyl-tRNA synthetase in order to investigate their conformational similarity. Since the conformations of aminoacyl adenylates examined were quite similar, the structure of isoleucyl adenylate was built and optimized to adopt their common conformation.
8. The structure of **18** was built and converted to a three-dimensional structure using the CONCORD program, Tripos. All molecular mechanics calculations were carried out with SYBYL 6.6, Tripos on a Silicon Graphics INDIGO 2 workstation.